PCT/GB2004/001408

INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed Anita Gerry

Dated 5 May 2004

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Patents Form 1/77

Patents Act 1977
(Rule 16)

The Patent Office

27MAR03 E795747-1 000016
P01/7700 0.00-0307079.4

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office
Cardiff Road
Newport
South Wales
NP9 1RH

1. Your reference — DB/STP-03132/BEP

2. Patent application number *(The Patent Office will fill in this part)* — 0307079.4 — 27 MAR 2003

3. Full name, address and postcode of the or of each applicant *(underline all surnames)* — STONE ISLAND HOLDINGS LTD
Half House, Military Road
Rye, East Sussex TN31 7NY

Patents ADP number *(if you know it)* — 82308 49001

If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom

4. Title of the invention — ALLICIN

5. Name of your agent *(if you have one)* — BROOKES BATCHELLOR

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)* — 102-108 CLERKENWELL ROAD
LONDON
EC1M 5SA

Patents ADP number *(if you know it)* — 08142291001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d)) — YES

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	13
Claim(s)	0
Abstract	0
Drawing(s)	2

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Brookes Batchellor Date 27/03/03

12. Name and daytime telephone number of person to contact in the United Kingdom DAVID BAILEY 01892 510600

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*

b) *Write your answers in capital letters using black ink or you may type them.*

c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*

d) *If you have answered 'Yes' Patents Form 7/77 will need to be filed.*

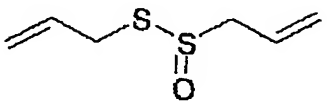
e) *Once you have filled in the form you must remember to sign and date it.*

f) *For details of the fee and ways to pay please contact the Patent Office.*

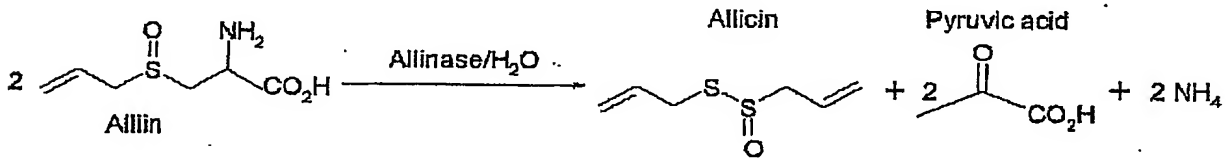
ALLICIN

The present invention relates to allicin.

Allicin, a sulphur compound having the formula:



is thought to be the principal active compound giving rise to the numerous therapeutic properties which are claimed for garlic (*Allium sativium*). In the natural state, garlic does not contain allicin, but a precursor, alliin [*(+) S-allyl-L-cysteine sulphoxide*]. Alliin is converted into allicin by the action of the enzyme *allinase* or *alliin lyase*, also a component of garlic. Alliin and allinase are brought together when garlic cloves are cut or crushed. The following equation represents the synthetic route.



However, allinase is rapidly and irreversibly deactivated by its reaction product, allicin, and is also deactivated in acid conditions such as the stomach. Thus, in practice, the yield of allicin from a clove of garlic falls far short of the theoretical maximum. Indeed, yields are usually of the order of 0.3-0.5%.

WO97/39115 describes a continuous process for the synthesis of allicin by preparing a column containing allinase immobilised on a solid support, passing a solution of alliin through the column and collecting a solution of allicin in the effluent.

Allicin is also prepared by the present applicant in liquid and spray-dried forms and is available in capsules and bulk powder form from Allicin International Limited of Half House,

Military Road, Rye, East Sussex, TN31 7NY, United Kingdom, under the trade mark ALLIMAX.

In our co-pending PCT application, WO03/024437, published today, we describe certain novel therapeutic properties of allicin.

The present invention is based on further investigations into therapeutic properties of allicin.

In one aspect, the present invention provides the use of allicin in the treatment of leishmaniasis. The present invention also provides the use of allicin in the preparation of a medicament for treatment of leishmaniasis. Preferably, allicin is present in the medicament at a concentration of about 5000 ppm.

In a second aspect, the present invention provides the use of allicin as a disinfectant or biocidal treatment of aquatic species. The present invention also provides the use of allicin in the preparation of a medicament for disinfection or biocidal treatment of aquatic species. Typically, the aquatic species are fish. This aspect of the present invention is particularly applicable to the fish farming and other aquatic or marine industries.

In a third aspect, the present invention provides the use of allicin as an antimicrobial agent for animal feed. Suitably the animal feed is water feed and allicin is present in an amount of about 500ppm. In an alternative embodiment, the animal feed is a feedstuff and allicin is present in an amount giving a daily intake of from 1 to 5 mg per animal per day. Suitably, for large animals such as cows or horses, allicin is present in an amount giving a daily intake of from 2.5 to 3 mg per animal per day. For smaller animals such as pigs or goats, allicin is present in an amount giving a daily intake of from 1.5 to 2.4 mg per animal per day.

In a fourth aspect, the present invention provides the use of allicin as a preservative agent in foodstuffs. The present invention also provides a food preservative agent comprising allicin and at least one food-grade excipient. Preferably, the preservative agent comprises allicin in a concentration of up to 500ppm.

In a fifth aspect, the present invention provides the use of allicin as a water disinfectant or biocide. The present invention also provides a water treatment composition comprising allicin and a food-grade excipient. In particular it provides such a water disinfectant or biocide for use in vegetable washing water, wastewater, stormwater or potable water treatments. Preferably, the water treatment composition comprises allicin in an amount of from 0.5 to 2.0 % w/v or w/w, more preferably in an amount of 0.9 to 1.7 %.

In a sixth aspect, the present invention provides the use of allicin as antiparasitic treatment for bees (apis). The present invention also provides the use of allicin in the preparation of an antiparasitic treatment for bees. The present invention also provides an antiparasitic treatment for bees comprising allicin and a pharmaceutically acceptable excipient. In particular, this aspect of the present invention provides a treatment against the Varroa mite and the bacteria Melissococcus plutonius (formerly called Streptococcus plutonius) and Paenibacillus larvae subsp. Larva and the fungal brood disease chalkbrood Ascophera apis.

In a seventh aspect, the present invention also provides the use of allicin in the preparation of a medicament for the treatment of Glycopeptide Intermediate Resistant Staphylococcus aureus.

Suitably for oral administration, or administration as a suppository, pessary or nasal preparation, the pharmaceutically acceptable excipient is a solid composition onto which the allicin or its metabolite is bound. More suitably, the solid composition comprises a bulking agent, such as lactose, microcrystalline cellulose or dicalcium phosphate, preferably cellulose; a thickening agent such as a gum or starch; a disintegrant, such as sodium starch glycolate or cross-linked povidone; a release agent such as magnesium stearate; an emulsifying agent; a surfactant and such sweeteners, fragrances and colorants as may be desired. Most preferably, allicin is bound by a spray drying process and the solid composition comprises a modified starch such as maltodextrin, gum acacia, silica and an emulsifier such as magnesium stearate.

WO02/062416 describes an apparatus for dispensing powdered material. It has been found that this apparatus is advantageous in delivery of a composition comprising allicin and a cellulose powder. Accordingly, in a final aspect of the present invention there is provided a composition comprising allicin and a cellulose powder.

Suitably, for topical application, the pharmaceutically acceptable excipient comprises a cream or a soap. The excipient may, alternatively, constitute a lotion, ointment, toothpaste, mouthwash or a hair preparation such as a shampoo, styling gel or conditioner. Such preparations may include a combination of the following as appropriate: surfactants, fragrances, colours, stabilisers, antioxidants, emulsifying agents, thickening agents, waxes, glycerols, fats, suspending agents, de- flocculating agents and antioxidants all of which may or may not be hypo-allergenic. Suitably, a cream excipient comprises white soft paraffin, an emulsifier such as a stearate, suitably magnesium stearate, glycerin, water, yellow soft paraffin and a stabiliser, such as potassium citrate. Most suitably, a cream excipient comprises an aqueous cream, preferably Aqueous Cream BP. Suitably, a soap excipient comprises ether sulphate, cocamide and cocobetaine. Optionally, the excipient may further include fragrances and colorants.

Suitably, for oral, parenteral and topical application, the ratio of allicin to excipient is such as to provide an allicin concentration of between 1ppm and 2000ppm, preferably between 50 and 1000ppm, more preferably between 250 and 500ppm.

The above and other aspects of the present invention will now be described in further detail, by way of example only.

THE USE OF ALLICIN IN THE TREATMENT OF LEISHMANIASIS

Leishmaniasis is a disease common in the tropics and sub-tropics caused by parasitic protozoans of the genus *leishmania* which are transmitted by the bite of sandflies. There are two principal forms of the disease - visceral leishmaniasis in which the cells of various internal organs are affected and cutaneous leishmaniasis which affects the tissues of the skin. This latter form itself has several different forms depending on the region in which it occurs and the protozooal species involved. Countries such as Panama, Honduras, the Amazon, South Central America and Asia are the areas where leishmaniasis is the most common.

In Asia for example, it is common in the form of an oriental sore and can be seen as a major third world problem. Leishmaniasis is a disease of the skin and mucous membranes resulting

in ulcerating lesions found on the arms and legs. The infection may also spread to the mucous membranes of the nose and mouth causing serious destruction of the tissues. Standard treatment is normally with drugs containing antimony but these are generally not readily available or well tolerated.

A form of leishmaniasis of the skin caused by the parasite leishmania tropica mexicana is also known as Chiclero's ulcer. The disease occurs in Panama, Honduras and the Amazon and primarily affects men who visit the forests to collect chicle (gum). This condition takes the form of an ulcerating lesion on the ear lobe and although the sore usually heals spontaneously within 6 months this can however cause a great deal of discomfort.

Confirmatory in vitro tests at the University of East London using allicin at a concentration of 5.0 gm per litre has killed the protozoal parasite associated with Leishmaniasis. Taken with extrapolation of the results from the laboratory studies described in PCT/GB2002/004309, we believe that allicin at a concentration of 5000ppm has efficacy as an antiprotozoal agent.

THE USE OF ALLICIN AS A DISINFECTANT/BIOCIDE IN FISH FARMING AND OTHER AQUATIC OR MARINE INDUSTRIES.

We have demonstrated that allicin can be used in fish farming and other aquatic industries to kill bacteria, parasites and fungi. Allicin can be used as an antimicrobial (including antibacterial, antiviral, antifungal and anti protozoal) preparation comprising allicin (and its metabolites, including DADS (Diallyldisulphide), DATS (Diallyltrisulphide), ajoene, allitridium and vinyldithiins).

Based on the test results from our laboratory studies on MRSA (30 strains), E.coli, E.Faecalis, Candida albicans, Pseudomonas aeruginosa, Salmonella typhimurium, Streptococcus pyogenes, B. subtilis, Serratia marcecens.etc, we believe that the results show that allicin can be used as an agent against bacteria and fungi.

Based on the results of our laboratory tests on lice (*Pediculus humanus)* contained in WO03/024437, we believe that allicin will destroy the parasites associated with fish farming and other aquatic or marine industries.

THE USE OF ALLICIN AS AN ANTIMICROBIAL AGENT IN ANIMAL FEED.

Allicin can be used as an antimicrobial agent in animal feed to promote growth in animals, prevent disease in animals and prevent the transmission of disease (including food poisoning) to humans. The antimicrobial (including antibacterial, antiviral, antifungal and anti protozoal) preparation comprises allicin (and its metabolites, including DADS (Diallyldisulphide), DATS (Diallyltrisulphide), ajoene, allitridium and vinyldithiins). Animals (for example, chickens, pigs, goats and cows) can pick up bacteria and pass these through the food chain to the human population. Conventional animal feedstuff and additives (including antibiotics) are used to prevent and treat disease in animals. Forthcoming European legislation suggests that the use of antibiotics may be banned or, at best, restricted.

TESTS AND DOSES

Our earlier application, WO03/024437, describes laboratory tests which show that allicin can kill E. coli, Listeria, E. Faecalis and other bacteria associated with animal diseases at a range of concentrations of up to 500ppm. By dosing the water feed channels of chickens with allicin at a concentration of 500ppm, allicin can be used as an antimicrobial preventative product. By dosing the feedstuff of animals such as pigs and goats with 1.5 mg to 2.4 mg of allicin per day, allicin can be used as an antimicrobial preventative product. By dosing the feedstuff of larger animals such as cows and horses with 2.5 mg to 3.0 mg of allicin per day allicin can be used as an antimicrobial preventative product.

THE USE OF ALLICIN AS A PRESERVATIVE AGENT IN FOOD PROCESSING.

Allicin can be used in food/meat processing to prevent the growth of bacteria that could cause and spread disease (including food poisoning) in humans, by means of an antimicrobial (including antibacterial, antiviral, antifungal and anti protozoal) preparations of allicin (and its

metabolites, including DADS (Diallyldisulphide), DATS (Diallyltrisulphide), ajoene, allitridium and vinyldithiins);

A range of concentrations of liquid allicin (0ppm to 500ppm) was applied to 10kg samples of hamburger meat to determine how long bacterial growth could be prevented. These tests were compared to the normal use of existing preservatives (including nitrates and phosphates).

To test for bacterial growth, small samples of meat were cut from the test piece of meat and, using standard methods of analysis, were checked for E.coli and Salmonella growth.

RESULTS

Allicin liquid with a concentration of 250ppm prevented bacterial overgrowth for up to 7 days.

Allicin liquid with a concentration of 375ppm prevented bacterial overgrowth for up to 10 days.

Allicin liquid with a concentration of 500ppm prevented bacterial overgrowth for up to 14 days.

A control sample of meat with no preservative or allicin showed strong bacterial growth after a few days.

Existing preservatives applied according to permitted normal practice prevented bacterial overgrowth for up to 7 days only.

The study demonstrated that allicin can be used as a preservative in food/meat processing. Standard methods of analysis demonstrated prevention of growth of E. coli and Salmonella at allicin concentrations of 250ppm (equivalent to 0.0250% w/v). Further evidence to demonstrate the preservative effect of allicin can be extrapolated from the test results of our laboratory studies on MRSA (30 strains), E.coli, E.Faecalis, F.streptococcus, Candida albicans, Pseudomonas aeruginosa, Salmonella typhimurium, Streptococcus pyogenes, B.

subtilis, Serratia marcecens, Listeria monocytogenes contained in PCT/GB2002/004309, confirming that allicin can be used as a preservative in food/meat preservation.

THE USE OF ALLICIN AS A DISINFECTANT/BIOCIDE IN VEGETABLE WASHING, WASTEWATER (including stormwater) TREATMENT AND DRINKING WATER TREATMENT.

Allicin can be used to displace or supplement existing harmful forms of disinfectant/biocide such as chlorine, sodium hypochlorite, ozone and per-acetic acid all of which can have an adverse effect on the environment. UV radiation is also used for disinfection but power and general running costs are high. Laboratory studies have been conducted on our behalf using allicin on aqueous suspensions of bacterial species commonly used as indicators of the effectiveness of water and wastewater disinfection. To this effect, identified isolates from the faecal coliform and streptococcus groups, namely, Escherichia coli (NCTC 8156) and Enterococcus hirae (University of Brighton isolate) were used in all experiments. An aqueous solution of allicin with a nominal concentration of allicin of 1.8 g per litre ie a 0.18% solution was used.

Stock suspensions of Escherichia coli (NCTC 8156) and Enterococcus hirae (University of Brighton isolate) were cultivated in Nutrient Broth No.2 from freeze dried isolates. Prior to each experiment, serial dilutions of the suspensions were enumerated by the spread plate method on Nutrient Agar and subsequent incubation at 37 deg C.

THE KELSEY-SYKES TEST

Initial experimental procedures were based on the methods set out in an established UK protocol (BS 6905: 1987). The Kelsy-Sykes methodology was developed as a guide to the concentrations of disinfectants which may be recommended for use under "dirty" (wastewater/sewage) conditions. It is therefore a suitable means of establishing the effectiveness of a disinfectant against a wastewater containing particulate and dissolved contaminants in addition to micro-organisms.

The basic Kelsy-Sykes test is used to establish the concentration of disinfectant and the contact time at which 3 out of 5 tubes demonstrate no growth of the test organism. It is not

designed to demonstrate the percentage kill of the test organism under any set of conditions. Therefore, with regard to wastewater/sewage considerations, the protocol was adapted. Under the revised methodology, a sample was taken from the bacterial suspension/biocide mix after the prescribed contact time and plated out onto solid media so that a colony count could be made (see Tables 1 and 2).

AGAR INHIBITION TESTS

This method was used to show the bactericidal effect of allicin and to study the zone of inhibition produced by the allicin solution on confluent growth of the test organisms on nutrient agar plates. Allicin solution concentrations of 100%, 50% 25% and 12.5% (in sterile distilled water) were pipetted into wells cored into Nutrient Agar plates on which E.coli isolate had been spread and cultured for 24 hours at 37 deg C. All plates were incubated for a further 24 hours at the same temperature and the zones of inhibition examined (see Plate 1).

RESULTS

Table 1 – percentage reduction in colony forming units of E.coli and Ent.hirae as a result of contact with allicin solutions in a modified Kelsey-Sykes test.

	Percentage reduction in colony forming units					
Allicin conc.	Escherichia coli			Enterococcus hirae		
%(w/v)	10 mins	20 mins	30 mins	10 mins	20 mins	30 mins
0.9	CG	CG	CG	CG	CG	CG
1.08	CG	CG	94	CG	CG	77
1.26	CG	CG	97	CG	CG	91
1.4	CG	CG	89	CG	CG	87
1.62	CG	CG	83	CG	CG	89

KEY: CG = Confluent growth

Table 2 – Numbers of colony forming units of E. coli and Ent. hirae killed as a result of contact with allicin solutions in a modified Kelsey-Sykes test.

Allicin conc. %(w/v)	Numbers of colony forming units killed					
	Escherichia coli			Enterococcus hirae		
	10 mins	20 mins	30 mins	10 mins	20 mins	30 mins
0.9	CG	CG	CG	CG	CG	CG
1.08	CG	CG	4.136x10E9	CG	CG	1.85x10E9
1.26	CG	CG	3.2x10E9	CG	CG	1.64x10E9
1.44	CG	CG	1.96x10E9	CG	CG	1.04x10E9
1.62	CG	CG	9.13x10E8	CG	CG	5.34x10E8

KEY: CG = Confluent growth

The study has demonstrated the bactericidal effect of allicin against bacteria commonly used as indicators of disinfection in water treatment. Simple tests on agar plates demonstrated inhibition of E. coli and Ent. Hirae at allicin concentrations as low as 0.225 g/l (equivalent to 0.0225% w/v). Further evidence to demonstrate the bactericidal effect of allicin on water-borne bacteria can be extrapolated from the test results of our laboratory studies on MRSA (30 strains), E.coli, E.Faecalis, F.streptococcus, Candida albicans, Pseudomonas aeruginosa, Salmonella typhimurium, Streptococcus pyogenes, B. subtilis, Serratia marcecens contained in our earlier patent application PCT/GB2002/004309.

THE USE OF ALLICIN AGAINST MITES AND BACTERIA THAT DESTROY BEES.

The Varroa mite is an indigenous parasite of honeybees (including Apis cerana and Apis mellifera). European foul brood disease is caused by a bacterium called Melissococcus plutonius (formerly called Streptococcus plutonius) which invades the mid-gut of four to five day old larvae. It multiplies rapidly in the mid-gut causing death. It only affects larvae in open brood. American foul brood disease is caused by Paenibacillus larvae subsp. Larva which affects the larvae in sealed brood cells. There is also a non-notifiable fungal brood disease called chalkbrood Ascophera apis which is a significant problem for some beekeepers.

Test results from our laboratory studies on MRSA (30 strains), E.coli, E.Faecalis, Candida albicans, Pseudomonas aeruginosa, Salmonella typhimurium, Streptococcus pyogenes etc; and other studies indicate that the liquid, cream and powder forms of allicin will destroy the Varroa mite, the European foul brood and the American foul brood bacteria.

EFFECTIVENESS OF ALLICIN AGAINST GLYCOPEPTIDE INTERMEDIATE RESISTANT STAPHYLOCOCCUS AUREUS

Staphylococcus aureus is the most common cause of community- and hospital-acquired infection in many areas of the world. In the 1980s, methicillin-resistant S. aureus (MRSA) emerged and became endemic in many hospitals. Vancomycin was the only antimicrobial agent with effective against some MRSA. In 1996, the first S. aureus strain with decreased susceptibility to vancomycin (glycopeptide intermediate-resistant S. aureus [GISA]) was reported in Japan. By 1997, the first GISA strains were reported in the United States and in 2003 a patient in the UK has died from an infection with a GISA strain. GISA strains can therefore cause serious morbidity and mortality.

Allicin in a liquid form has been tested against the GISA strain isolated from the UK mortality. In a standard agar diffusion test the strain produced a zone of 37mm at 500ppm (Plate 2) and 30mm at 300ppm. The GISA strain was therefore fully susceptible to allicin at our recommended doses for topical use.

DELIVERY OF ALLICIN AND THE APPARATUS OF WO02/062416

Preparations of allicin and cellulose have been prepared both with and without additional pharmaceutically acceptable excipients. The preparation was delivered to the target areas by the dry spray device of WO02/062416. WO02/062416 describes the use of the apparatus for delivering cellulose to the nasal tract for the treatment of hayfever. This apparatus allows the combination of allicin powder and cellulose to be sprayed by the individual patient onto the target areas (including the nasal tract). In order to test this novel method of delivering allicin to the target areas, mixtures of allicin powder with the cellulose powder provided by the applicant company of WO02/062416, Nasaleze Ltd, were investigated for anti-staphylococcal activity.

The biological activity of allicin against bacteria is well established. In studies contained in our earlier patent application, WO03/024437, we have already shown that certain species of methicillin resistant *Staphylococcus aureus* (MRSA) are exceptionally susceptible to allicin.

Using a susceptible strain of MRSA, we have developed a novel method whereby we can determine whether or not different batches of allicin possess biological activity.

There are a number of tests available to determine the anti-microbial activity of selected agents. Diffusion tests determine the susceptibility of isolates by measuring the zones of inhibition around a measured amount of the anti-microbial agent. Zones of inhibition not more than 6mm smaller than those of a known control strain indicate that the test bacterium is sensitive to the anti-microbial agent. Zone sizes of 12mm or less usually indicate antibiotic resistance. There is also an intermediate antibiotic resistant group between with susceptibilities between these levels and zone sizes greater than 12mm.

Materials and methods

Bacteria: MRSA clinical isolate UEL301 was used. Overnight broth cultures in isosensitest broth were prepared.

Media: Isosensitest agar (Oxoid Ltd) were used.

Powders: supplied by Allicin International (cellulose powder from Nasaleze Ltd + allicin powder)

Method:

- A broth containing 10^5 cfu/ml was prepared in peptone water.
- 0.2ml was spread over each isosensitest plate.
- Plates were air dried and a 6mm well cut in the centre of the plate.
- A volume of 100ug or 150ug of each powder was added to each well.
- Plates were incubated overnight at 37 deg C.
- The presence of zones of inhibition around a well is indicative of biological activity being present. No zone around the 6mm well, (as with the negative control) represented no biological activity.

The following ratios of allicin powder and cellulose were used:

Allicin Powder : Cellulose Powder = 2:1, 4:1, 6:1 and 8:1.

Tests were also carried out using allicin powder alone, cellulose powder alone and gum acacia powder alone. The concentration of allicin in the allicin powder was nominally 250ppm.

Results

COMPARATIVE ZONE SIZES IN MM (0 represents 6mm well size)

Number	Preparation	100ug	Bioactive	150ug	Bioactive
1	Negative control	0 (6mm)	-	0 (6mm)	-
2	Nasaleze (cellulose powder)	0	-	0	-
3	Allicin CPC 2069/03	14	+	19	+
4	Allicin CPC 2102 4-1	23	+	27	+
5	Allicin CPC 2102 6-1	28	+	28	+
6	Allicin CPC 2069/03 4-1	12	+	17	+
7	Allicin CPC 2102 8-1	22	+	26	+

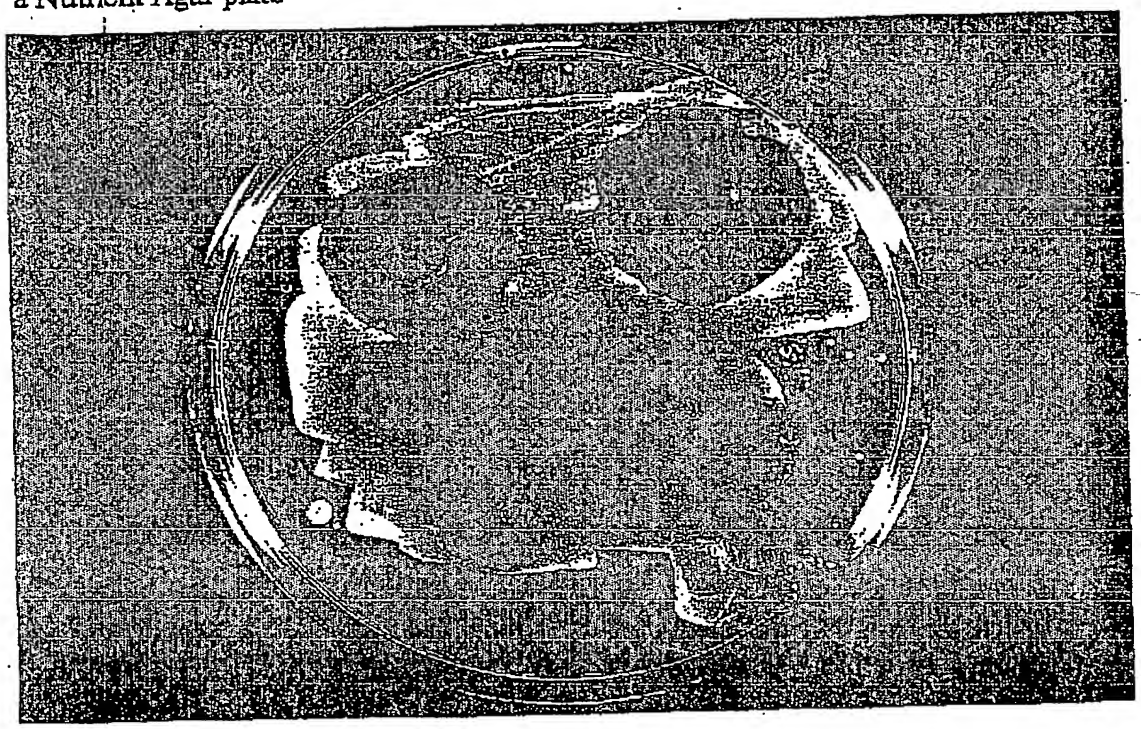
The gum acacia alone showed minimal antibacterial activity yielding a zone of 2 or 3 mm. The cellulose powder alone showed no bacterial activity.

Therefore, the above tests demonstrate the antimicrobial activity of a number of allicin/cellulose powder mixtures (delivered by the apparatus of WO02/004309 or similar vehicles for delivery of powdered materials) against MRSA and other multiply drug resistant bacteria including MDRTB (Multiply drug resistant tuberculosis), VRSA (Vancomycin resistant *Staphylococcus aureus*), MRSE (methicillin resistant *Staphylococcus epidermidis*), PRSP (Penicillin resistant *Streptococcus pneumoneae*), VRE (Vancomycin resistant enterococci) and VISA (Vancomycin intermediate resistant *Staphylococcus aureus*).

1/2

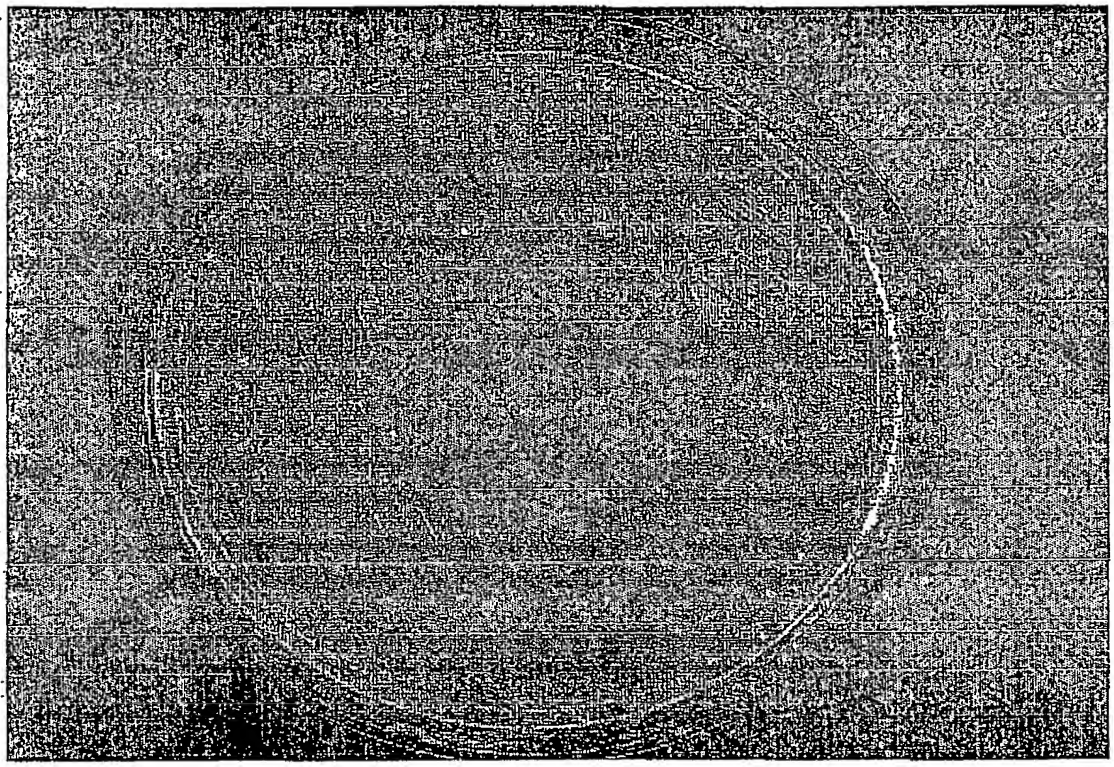
PLATE 1

~~Figure 3.1~~ Zones of inhibition of *Escherichia coli* around wells of allicin solution on a Nutrient Agar plate



Anticlockwise from top right 100%, 50%, 12.5%, and 25% dilutions of original allicin solution (1.8% w/v).

Plate 2 Zone of inhibition produced by 500ppm of allicin against a GISA strain.



BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ BLACK BORDERS
- ☒ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☒ BLURED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLORED OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REPERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning documents *will not* correct images problems checked, please do not report the problems to the IFW Image Problem Mailbox